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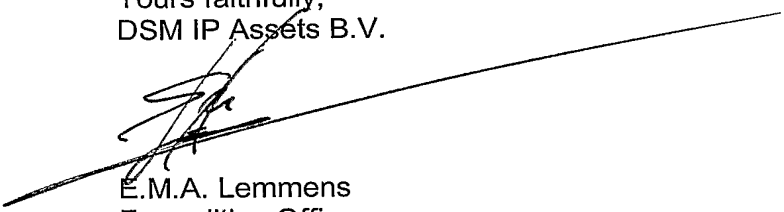
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We enclose the following document(s) in connection with the above patent application:

– Priority Document

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Patentanmeldung Nr.    Patent application No.    Demande de brevet n°

04075597.7

Der Präsident des Europäischen Patentamts;  
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets  
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R C van Dijk





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Application no.: 04075597.7  
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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:  
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.  
If no title is shown please refer to the description.  
Si aucun titre n'est indiqué se référer à la description.)

Process for the enzymatic preparation of an enantiomerically enriched beta-2-amino acid(ester)

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s)  
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PROCESS FOR THE ENZYMATIC PREPARATION OF AN ENANTIOMERICALLY  
ENRICHED BETA-2-AMINO ACID (ESTER)

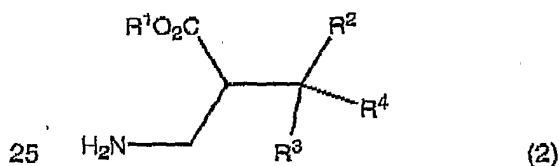
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The invention relates to an enzymatic process for the preparation of an enantiomerically enriched  $\beta^2$ -amino acid (ester).

An enzymatic process for the preparation of an enantiomerically enriched  $\beta^2$ -amino acid (ester) is unknown. Salamonczyk, G.M. *et al. J. Org. Chem.*, (1996), vol. 61, 6893-6900 discloses a slightly related enzymatic process to a different compound in which an enantiomerically enriched 3-amino-2-methyl-propionic acid is prepared by reacting racemic N-protected 3-amino-2-methyl-propionic acid methyl ester with immobilized *Candida Antarctica* lipase. However, this process relates to N-protected  $\beta^2$ -amino acids and N-protected  $\beta^2$ -amino acid esters. Furthermore, a disadvantage of this process is that it is necessary to use a multistep kinetic resolution in order to get an acceptable enantiomeric excess of 3-amino-2-methyl-propionic acid.

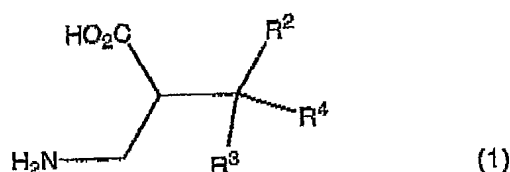
It is the object of the invention to provide a simple enzymatic process for the preparation of an enantiomerically enriched  $\beta^2$ -amino acid (ester), i.e. a process without the need of multistep kinetic resolutions and/or N-protection of the  $\beta^2$ -amino acid (ester).

This object is achieved according to the invention by reacting a stereoselective hydrolytic enzyme with a mixture of enantiomers of a  $\beta^2$ -amino acid ester represented by formula 2



wherein  $R^1$  stands for an optionally substituted alkyl and wherein  $R^2$ ,  $R^3$  and  $R^4$  each independently stand for H, an optionally substituted (hetero)aryl, an optionally substituted alkyl,  $OR^5$ ,  $CO_2R^6$ ,  $C(O)R^7$ ,  $SR^8$ ,  $NR^9R^{10}$ ,  $OC(O)R^{11}$  wherein  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^8$ ,  $R^9$ ,  $R^{10}$  and  $R^{11}$  each independently stand for H, an optionally substituted alkyl or for an optionally substituted (hetero)aryl and wherein  $R^2$  and  $R^3$ ,  $R^2$  and  $R^4$  or  $R^3$  and  $R^4$  may form a ring together with the carbon atom to which they are attached and by either collecting the resulting enantiomerically enriched  $\beta^2$ -amino acid of formula 1





5 , wherein  $R^2$ ,  $R^3$  and  $R^4$  are as defined above or by collecting the remaining enantiomerically enriched  $\beta^2$ -amino acid ester of formula 2.

Surprisingly the process of the invention is a simple process, which does not require multistep kinetic resolutions and/or N-protection of the  $\beta^2$ -amino acid (ester), which process leads to  $\beta^2$ -amino acid (ester)s with an acceptable enantiomeric excess. Furthermore, the process of the invention is well scalable and can be applied  
10 at an industrial scale.

With  $\beta^2$ -amino acid (ester) is meant a  $\beta$ -amino acid(ester) substituted on the 2-position ( $\alpha$ -position). For a definition of  $\beta^2$ -amino acid(ester) see also Sewald, Angew. Chem. Int. Ed. (2003), 42, 5794-5795.

The  $\beta^2$ -amino acid (ester) of formula 2 has a chiral center on at least  
15 the  $\alpha$ -position; the  $\beta^2$ -amino acid (ester) of formula 2 may however also have other chiral centers, for example the carbon atom to which  $R^2$ ,  $R^3$  and  $R^4$  are attached may be chiral; this carbon atom is chiral when  $R^2$ ,  $R^3$  and  $R^4$  are not the same.

With 'mixture of enantiomers' is meant a random mixture of (*R*) and (*S*)-enantiomers. Typically, a racemic mixture of  $\beta^2$ -amino acid esters of formula 2 is  
20 used (i.e. when (*R*): (*S*) is 1:1), but of course the process of the invention may also be performed, - for further enantiomeric enrichment -, on an already enantiomerically enriched mixture of enantiomers

Preferably,  $R^1$  stands for an optionally substituted alkyl of 1-20 C-atoms, more preferably of 1-12 C-atoms (C-atoms of the substituents included). For  
25 example  $R^1$  may stand for a  $C_1$ - $C_4$  alkyl, e.g. methyl, ethyl, *n*-butyl; substituted methyl, e.g. benzyl; or substituted ethyl, e.g. trichloroethyl, methoxy-ethyl or trifluoroethyl.

Preferably,  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^8$ ,  $R^9$ ,  $R^{10}$  and  $R^{11}$  each independently stand for H, an optionally substituted alkyl of 1-12 C-atoms, more preferably of 1-8 C-atoms (C-atoms of the substituents included) or for a (hetero)aryl of  
30 2-10 C-atoms (C-atoms of the substituents included). Preferably in the heteroaryl, the heteroatom(s) is/are chosen from the group of N, O and S.

$R^2$  and  $R^3$ ,  $R^2$  and  $R^4$  or  $R^3$  and  $R^4$  may form a ring of preferably 3-6 C-atoms. The ring they form may for example be a (hetero)cycloalkyl or a (hetero)aryl,

each of which rings may optionally be substituted.

Examples of substituents include alkyl, (hetero)aryl, sulfonyl, alkoxy carbonyl, amidocarbonyl, nitrile, hydroxy, alkoxy, aryloxy, thioalkyl, mercapto, amino and fluorine.

5 In the framework of the invention with the term 'enantiomerically enriched' is meant 'having an enantiomeric excess (e.e.) of either the (*R*)- or (*S*)- enantiomer of a compound'. Preferably, the enantiomeric excess is > 80%, more preferably > 85%, even more preferably > 90%, in particular > 95%, more in particular > 97%, even more in particular > 98%, most in particular > 99%.

10 Collecting includes for example isolation by means of conventional methods, for example ultrafiltration, concentration, column chromatography, extraction or crystallization and further reaction of the obtained product (enantiomerically enriched  $\beta^2$ -amino acid or enantiomerically enriched  $\beta^2$ -amino acid ester).

With 'hydrolytic enzyme' is meant an enzyme with the ability to  
15 hydrolyze a carboxylic ester group to form the corresponding carboxylic acid group.

With 'stereoselectivity' of the hydrolytic enzyme is meant that the hydrolytic enzyme preferably catalyzes the hydrolysis of one of the enantiomers of the  $\beta^2$ -amino acid ester. The stereoselectivity of an enzyme may be expressed in terms of E-ratio, the ratio of the specificity constants  $V_{max}/K_m$  of the two enantiomers as  
20 described in C-S. Chen, Y Fujimoto, G. Girdaukas, C. J. Sih., *J. Am. Chem. Soc.* 1982, 104, 7294-7299. Preferably, the hydrolytic enzyme has an E-ratio > 5, more preferably an E-ratio > 10, even more preferably an E-ratio > 50, most preferably an E-ratio > 100.

A stereoselective hydrolytic enzyme suitable for use in the present invention may for example be found in one of the general classes of hydrolytic  
25 enzymes, for instance in the group of esterases, lipases, proteases, peptidases or acylases, preferably in the group of esterases or lipases. The hydrolytic enzyme may be derived from both eukaryotic and prokaryotic cells, including but not limited to those from the following mammalian sources: porcine liver, porcine pancreas, for example commercially available porcine pancreatic lipase type II (L-3126, Sigma); porcine  
30 kidney and bovine pancreas; those from the plant source wheat germ; those from the following mold genera: *Absidia*; *Aspergillus*; *Fusarium*; *Gibberella*; *Mucor*; *Neurospora*; *Trichoderma*; *Rhizopus*; *Rhizomucor*, for example *Rhizomucor miehei*; *Thermomyces*, for example *Thermomyces lanuginosus*; those from the following bacterial genera: *Achromobacter*; *Alcaligenes*; *Bacillus*; for example *Bacillus licheniformis*;  
35 *Brevibacterium*; *Corynebacterium*; *Providencia*; *Pseudomonas*, for example *Pseudomonas fluorescens*, *Pseudomonas cepase* or *Pseudomonas alcaligenes*; *Serratia*; *Rhodococcus*, those from the following yeast genera: *Candida*, for example

*Candida rugose* or *Candida Antarctica*; and those from the Actinomycete genus *Nocardia*.

Preferably, the stereoselective hydrolytic enzyme is found in the group of enzymes classified as carboxylic ester hydrolases (EC 3.1.1) or in the group of enzymes classified as peptidases, for example EC 3.4.1, EC 3.4.11, EC 3.4.21, more preferably EC 3.4.21.62, EC 3.4.22 or EC 3.4.23.

A stereoselective hydrolytic enzyme may also be found in the group of commercially available hydrolytic enzymes. Examples of commercially available hydrolytic enzymes are: enzymes supplied by Fluka: *Candida cylindracea* lipase, lipase Hog pancreas, lipase *Pseudomonas fluorescens*, lipase *Aspergillus oryzae*, lipase *Rhizopus niveus*, lipase *Rhizomucor miehei*, lipase *Candida antarctica*, lipase *Mucor javanicus*, lipase *Rhizopus arrhizus*, lipase *Penicillium roqueforti*, lipase *Candida lipolytica*, lipoprotein lipase *Pseudomonas* sp., type B, lipoprotein lipase *Pseudomonas cepacia*, lipoprotein lipase *Chromobacterium viscosum*, esterase *Bacillus stearothermophilus*, esterase *Bacillus thermoglucosidasius*, esterase *Mucor miehei*, esterase hog liver; enzymes supplied by Altus: *Candida rugosa* lipase, lipase *Mucor miehei*, *Candida antarctica* B lipase, *Candida antarctica* A lipase, Chiro-CLEC-CR, Chiro-CLEC-CR (slurry), porcine liver esterase, penicillin acylase, subtilisin Carlsberg, Chiro-CLEC-BL (slurry), Chiro-CLEC-PC (slurry), Chiro-CLEC-EC (slurry), *Aspergillus oryzae* protease, PeptiCLEC-TR (slurry); enzymes supplied by Recombinant Biocatalysis: ESL-001-07, ESL001-01, ESL-001-01 with stabilizer, ESL-001-02, ESL-001-03, ESL-001-05; enzymes supplied by Boehringer-Mannheim: Chirazyme L4 (*Pseudomonas* sp.), Chirazyme L5 (*Candida antarctica* fraction A), Chirazyme L1 (*Burkholderia*), Chirazyme L6 (porcine pancreas), Chirazyme L7, Chirazyme L8; enzymes supplied by DSM (formerly Gist-Brocades): Naproxen esterase, Lipomax, Genzyme, Lipoprotein lipase; enzymes supplied by Novo: Novozyme 868, Novozyme 435, immobilized *Candida Antarctica* lipase, Nagase enzyme, Lipase A-10FG (*Rhizopus javanicus*); enzymes supplied by Amano, Amano AYS, Amano PS, Amano PSD, Amano AKD11, Amano AKD111.

The most preferred stereoselective hydrolytic enzymes are: subtilisin, alcalase 2.5L, *Bacillus licheniformis* protease, *Candida Antarctica* lipase A, porcine pancreatic lipase type II.

The stereoselective hydrolytic enzyme may be used in any form. For example, the hydrolytic enzyme may be used - for example in the form of a dispersion, a solution or in immobilized form - as crude enzyme, as a commercially available enzyme, as an enzyme further purified from a commercially available preparation, as an enzyme obtained from its source by a combination of known purification methods, in

whole (optionally permeabilized and/or immobilized) cells that naturally or through genetic modification possess the required stereoselective hydrolytic enzyme activity, or in a lysate of cells with such activity.

It will be clear to the average person skilled in the art that use can also be made of mutants of naturally occurring (wild type) enzymes with hydrolytic activity in the process according to the invention. Mutants of wild-type enzymes can for example be made by modifying the DNA encoding the wild type enzymes using mutagenesis techniques known to the person skilled in the art (random mutagenesis, site-directed mutagenesis, directed evolution, gene shuffling, etc.) so that the DNA encodes an enzyme that differs by at least one amino acid from the wild type enzyme and by effecting the expression of the thus modified DNA in a suitable (host) cell. Mutants of the stereoselective hydrolytic enzyme may have improved properties with respect to (stereo)selectivity and/or activity and/or stability and/or solvent resistance and/or pH profile and/or temperature profile.

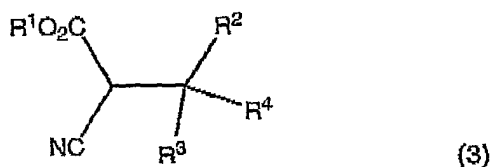
The choice of the reaction conditions of the process of the invention depends on the choice of hydrolytic enzyme. Usually, the temperature of the process is chosen between 0 and 90°C, in particular between 10 and 40°C; usually the pH of the process is chosen between 4 and 10 and as a solvent usually water, an aqueous solvent, for example water/*t*-butanol or water/dioxane, or a two-phase system of water and a water immiscible solvent, for example toluene, hexane, heptane, methyl *t*-butyl ether, methyl iso-butyl ketone is used.

The resulting enantiomerically enriched  $\beta^2$ -amino acid of formula 1 obtained in the process of the invention may be isolated by using a known method for the isolation of an acid from water or from an aqueous layer. The remaining enantiomerically enriched  $\beta^2$ -amino acid ester of formula 2 obtained in the process of the invention may also be isolated in a manner known per se, for example by extraction with an organic solvent, crystallization or (ion exchange) chromatography.

Optionally, the collected remaining enantiomerically enriched  $\beta^2$ -amino acid ester of formula 2 is further hydrolysed to form the corresponding enantiomerically enriched  $\beta^2$ -amino acid. Methods for the hydrolysis of an ester are known to the person skilled in the art, for example esters may be hydrolysed by acid hydrolysis using an aqueous solution of a mineral acid solution, for example HCl or H<sub>2</sub>SO<sub>4</sub>, by saponification using an aqueous solution of sodium hydroxide, or by enzymatic hydrolysis.

A mixture of enantiomers of  $\beta^2$ -amino acid esters of formula 2, wherein R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are as defined above may be prepared using methods

known in the art. For example, a  $\beta^2$ -amino acid ester of formula 2 may be prepared by reduction of the corresponding nitrile of formula 3

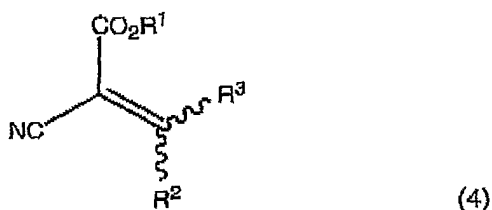


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wherein  $R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$  are as defined above with a suitable reducing agent and optionally in the presence of a suitable catalyst.

The nitrile of formula 3, wherein  $R^1$ ,  $R^2$  and  $R^3$  are as defined above and wherein  $R^4$  stands for H may be prepared by reduction of the double bond of the corresponding nitrile of formula 4,

10



wherein  $R^1$ ,  $R^2$  and  $R^3$  are as defined above with a suitable reducing agent and optionally in the presence of a suitable catalyst.

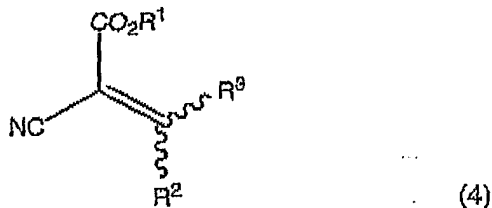
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In one embodiment of the invention, the mixture of enantiomers of  $\beta^2$ -amino acid esters of formula 2, wherein  $R^1$ ,  $R^2$  and  $R^3$  are as defined above and  $R^4$  stands for H may be prepared by a one-pot reduction (this is: without isolation of the intermediate nitrile of formula 3) of the corresponding nitrile of formula 4 with a suitable reducing agent and optionally in the presence of a suitable catalyst. In this reduction both the double bond and the nitrile group of the compound of formula 3 are reduced.

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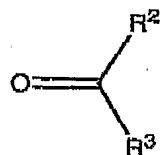
Suitable reducing agents (and catalyst combinations) are known to the person skilled in the art and include for example  $H_2$  with a Pd/carbon catalyst,  $H_2$  with Raney Nickel,  $H_2$  with Pt/carbon,  $H_2$  with Ru/carbon and the like. For example the nitrile of formula 3, wherein  $R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$  are as defined above may be prepared from the corresponding nitrile of formula 4

25



wherein  $R^1$ ,  $R^2$  and  $R^3$  are as defined above in a manner known per se by introducing  $R^4$  via nucleophilic 1,4-addition using a suitable nucleophile. The person skilled in the art knows which nucleophiles to use to introduce the desired  $R^4$ . Examples of suitable nucleophiles include alkyl lithium, for example methyl lithium; Grignard reagents; alkoxides, for example a salt of benzylalcohol, for instance the potassium salt; thiols, for example thioacetic acid or benzylmercaptan, both of which may be in the form of a salt; nitrogen nucleophiles, for example amines, for example hydroxylamine; azides; or imides, for example potassium phthalimide or potassium bisformimide.

- 10 A nitrile of formula 4 wherein  $R^1$ ,  $R^2$  and  $R^3$  are as defined above may also be prepared by condensation of a ketone or aldehyde of formula 6



(6)

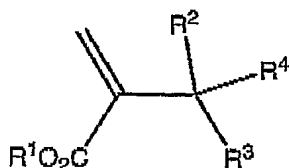
- 15 wherein  $R^2$  and  $R^3$  are as defined above and a nitrile of formula 7



(7)

- 20 wherein  $R^1$  is as defined above, in the presence of a suitable base or a dehydrating reagent. Suitable bases and dehydrating reagents are well known in the art. Suitable bases, include for example piperidine, triethylamine,  $OH^-$ , diazabicycloundecene (DBU) and diazabicyclononene (DBN). An example of a dehydrating reagent is ammoniumacetate.

- 25 A mixture of enantiomers of  $\beta^2$ -amino acid esters of formula 2, wherein  $R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$  are as defined above may, for example, also be prepared by reacting  $NH_3$  or an  $NH_3$ -analogue with the 2-substituted acrylic acid ester of formula 5



(5)

- 30 wherein  $R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$  are as defined above.

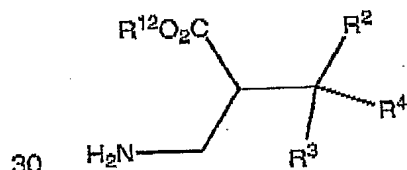
With an  $\text{NH}_3$ -analogue is meant a compound which will give the free amine in the compound of formula 2 (optionally after removal of a protecting group). Examples of  $\text{NH}_3$ -analogues include benzylamine, dibenzylamine, azide, hydroxylamine, potassium phthalimide and potassium bisformimide.

5 Enantiomerically enriched  $\beta^2$ -amino acids and enantiomerically enriched  $\beta^2$ -amino acid esters may be used as building blocks in the preparation of pharmaceuticals and agrochemicals. For example,  $\beta^2$ -homo-threonine can be used in the preparation of the pharmaceutically active ingredient of  $\beta$ -lactam antibiotics (EP290385, EP742223, EP371875, EP774463, C Fuganti *et al.* Org. Bio-Org.Chem  
10 (1993), 2247-2249).  $\beta^2$ -amino acids may for example also be used in the preparation of the pharmaceutically active ingredient of platelet aggregation inhibitors (US 5,344,957, WO93/07867) or in the preparation of the pharmaceutically active ingredient of enkephalinase inhibitors (to replace morphine, treatment of diarrhea, hypertension, cardiac insufficiency and as analgesics; EP 634396). Furthermore,  $\beta^2$ -amino  
15 acids/esters may also be used to prepare pharmaceutically active ingredients useful as an antibiotic (WO02/41886) or as a medicine against constipation (Drugs Fut. (2000), 25(12), p1308).

The invention therefore, also relates to a process wherein the enantiomerically enriched  $\beta^2$ -amino acid (ester) prepared according to the invention is  
20 further converted into a pharmaceutically active ingredient in a manner known per se.

Furthermore, the invention also relates to a process wherein the pharmaceutically active ingredient as obtained above is formulated into a pharmaceutical composition comprising the pharmaceutically active ingredient and  
25 excipient.

The stereoselective hydrolytic enzyme suitable for use in the present invention may also be used in the preparation of an enantiomerically enriched  $\beta^2$ -amino acid ester of formula (2), wherein  $\text{R}^1$ ,  $\text{R}^2$ ,  $\text{R}^3$  and  $\text{R}^4$  are as defined above from an enantiomerically enriched  $\beta^2$ -amino acid ester of formula (8)



(8)

wherein  $\text{R}^1$  stands for an optionally substituted alkyl, but is not the same as  $\text{R}^1$ , and an alcohol  $\text{R}^1\text{OH}$ , wherein  $\text{R}^1$  is as defined above.

Preferably, R<sup>1</sup> stands for an optionally substituted alkyl of 1-20 C-atoms, more preferably of 1-12 C-atoms (C-atoms of the substituents included). For example R<sup>1</sup> may stand for a C<sub>1</sub>-C<sub>4</sub> alkyl, e.g. methyl, ethyl, *n*-butyl; substituted methyl, e.g. benzyl; or substituted ethyl, e.g. trichloroethyl, methoxy-ethyl or trifluoroethyl.

5 Preferably the molar amount of alcohol R<sup>1</sup>OH relative to the amount of amino acid ester of formula 8 is >5, more preferably > 10.

The invention will now be elucidated by way of the following examples without however being limited thereto.

## 10 Examples

### Example 1. Preparation of (±)-3-amino-2-benzyl-propionic acid methyl ester hydrochloride.

To a mixture of methyl cyanoacetate (50 g, 0.5 mol) and benzaldehyde (53.5 g, 0.5  
15 mol) in dioxane (150 mL) is added acetic acid (2.9 mL, 0.05 mol), piperidine (2.0 mL, 0.02 mol) and palladium on carbon 5 wt% (2 g). The mixture is stirred under hydrogen (1-2 bar) at room temperature for 16 hours. Then the reaction mixture is filtered on celite and the filtrate is evaporated to dryness. The residue is redissolved in ethyl acetate (200 mL) and washed with water and brine. The organic layer is dried on  
20 sodium sulphate, filtered and evaporated to dryness to afford a yellow oil (96 g, quantitative yield). To the solution of this oil (50 g, 0.25 mol) in methanol (300 mL) is added palladium on carbon 5 wt% (1 g) and HCl 37 wt% (33 mL, 0.4 mol) and the reaction mixture is stirred under 10 bar of hydrogen. After 17 hours of stirring, the solution is filtered on celite, evaporated to dryness and the residue is precipitate in  
25 ether to afford a white powder of (±)-3-amino-2-benzyl-propionic acid methyl ester hydrochloride in 61% yield.

<sup>1</sup>H-NMR (400MHz, DMSO) δ 8.49 (s, 3H), 7.19-7.30 (m, 5H), 3.58 (s, 3H), 3.11-3.21 (m, 1H), 2.81-3.05 (m, 4H).

## 30 Example 2

### Preparation of (±)-2-(aminomethyl)-4-methyl-pentanoic acid methyl ester hydrochloride.

To a mixture of methyl cyanoacetate (50 g, 0.5 mol) and isobutyraldehyde (36 g, 0.5  
35 mol) in dioxane (150 mL) is added acetic acid (2.9 mL, 0.05 mol), piperidine (2.0 mL, 0.02 mol) and palladium on carbon 5 wt% (2 g). The mixture is stirred under hydrogen (1-2 bar) at room temperature for 18 hours. Then the reaction mixture is filtered on



- celite and the filtrate is evaporated to dryness. The residue is redissolved in ethyl acetate (200 mL) and washed with water and brine. The organic layer is dried on sodium sulphate, filtered and evaporated to dryness to afford a yellow oil (78 g, quantitative yield). To the solution of this oil (40 g, 0.26 mol) in methanol (300 mL) is
- 5 added palladium on carbon 5 wt% (1 g) and HCl 37 wt% (32 mL, 0.38 mol) and the reaction mixture is stirred under 10 bar of hydrogen. After 17 hours of stirring, the solution is filtered on celite, evaporated to dryness and the residue is precipitate in ether to afford a white powder of  $(\pm)$ -2-(aminomethyl)-4-methyl-pentanoic acid methyl ester hydrochloride in 57 % yield.
- 10  $^1\text{H-NMR}$  (400MHz, DMSO)  $\delta$  8.24 (s, 3H), 3.67 (s, 3H), 2.81-3.09 (m, 3H), 1.25-1.58 (m, 3H), 0.98 (d, 6H).

### Example 3

#### Preparation of $(\pm)$ -2-(aminomethyl)-3-methyl-butyrac acid methyl ester hydrochloride.

- 15 To a mixture of methyl cyanoacetate (10 g, 0.1 mol) and acetone (6.38 g, 0.11 mol) in ethanol (20 mL) is added acetic acid (1.2 g, 0.02 mol), ammonium acetate (0.78 g, 0.01 mol) and palladium on carbon 5 wt% (0.4 g). The reaction mixture is stirred at room temperature under hydrogen (1-2 bar) for 18 hours. After filtration on celite, the filtrate
- 20 is evaporated and the residue is solubilised in ethyl acetate and washed with water and brine. The organic layer is dried over sodium sulphate and then concentrated in vacuo to afford a yellow oil (30 g). To a solution of this yellow oil in methanol (200 mL) is added palladium on carbon 5 wt% (1 g) and HCl 37 wt% (10.5 mL, 0.15 mol). After 15 hours stirring under hydrogen (10 bar), the reaction mixture is filtered on celite and the
- 25 filtrate is concentrated in vacuo. The 2-(aminomethyl)-3-methyl-butyrac acid methyl ester hydrochloride is obtained in 43% yield after precipitation in acetone.
- $^1\text{H-NMR}$  (400MHz, DMSO)  $\delta$  8.18 (s, 3H), 3.67 (s, 3H), 3.00 (m, 2H), 2.65 (m, 1H), 1.97 (m, 1H), 0.90 (m, 6H).

### 30 Example 4

#### Preparation of $(\pm)$ -2-(aminomethyl)-butyrac acid methyl ester hydrochloride.

- To a mixture of methyl cyanoacetate (10 g, 0.1 mol), acetaldehyde (6.76 mL, 0.12 mol) and palladium on carbon 5%wt (0.4g) in acetic acid (16mL) is added slowly a solution
- 35 of piperidine (0.34 g, 0.04 mol) in acetic acid (4 mL). The reaction mixture is stirred at room temperature under hydrogen (1-2 bar) for 17 hours then filtered on celite and

concentrated to afford a yellow oil (12 g). To the solution of this oil in methanol (150 mL) is added palladium on carbon 5%wt (1.2 g) and HCl 37 wt% (12 mL, 0.15 mol). The reaction mixture is stirred at room temperature under hydrogen (10 bar) for 15 hours and the filtered and concentrated in vacuo to afford the 2-(aminomethyl)-butyric acid methyl ester hydrochloride in 47% yield after precipitation in ether.

5 <sup>1</sup>H-NMR (400MHz, DMSO)  $\delta$  8.24 (s, 3H), 3.67 (s, 3H), 2.81-3.09 (m, 2H), 2.58-2.71 (m, 1H), 1.61 (q, 2H), 0.98 (t, 3H).

#### Example 5

10 Preparation of (+)-3-amino-2-methyl-propionic acid methyl ester, acetic acid salt.

A solution of methacrylic acid methyl ester (10 g, 0.1 mol) and benzylamine (10.7 g, 0.1 mol) is refluxed in methanol (100 mL) for 4 days, then the solvent is evaporated to afford the 3-benzylamino-2-methyl-propionic acid methyl ester in 100% yield. To a solution of this ester (4 g, 19.3 mmol) in methanol (70 mL) is added palladium on carbon 5 wt% (0.4 g) and acetic acid (2.2 mL, 38 mmol). The solution is stirred for 5 hours under 1-2 bar of hydrogen, then filtered on celite and concentrated in vacuo to afford the 3-amino-2-methyl-propionic acid methyl ester, acetic acid salt in 85% yield.

15 <sup>1</sup>H-NMR (400MHz, DMSO)  $\delta$  8.72 (s, 3H), 3.64 (s, 3H), 3.09 (m, 1H), 2.81 (m, 2H), 2.31 (s, 3H), 1.15 (d, 3H).

20

#### Example 6

Preparation of (S)-3-amino-2-benzyl-propionic acid methyl ester and preparation of (R)-3-amino-2-benzyl-propionic acid.

25 To a solution of 3-amino-2-benzyl-propionic acid methyl ester hydrochloride (10 g, 44 mmol) in 100 mL of potassium phosphate buffer (25 mM, pH 7.0) is added Alcalase type B 2.5 enzyme (1.25 g). The resulting mixture is stirred at room temperature and the pH is adjusted to pH 7.0 during the reaction with a 2N NaOH aqueous solution. The reaction is stopped at 50% conversion after 2h. The reaction mixture is filtered, the pH is adjusted to pH 9 with solid K<sub>2</sub>CO<sub>3</sub> and the remaining unreacted ester is removed by extraction (3 times) with methylene chloride. The organic layer is dried over sodium sulphate, filtered and concentrated in vacuo to afford the enantiomerically enriched (S)-3-amino-2-benzyl-propionic acid methyl ester (ee>99%). The aqueous layer is slowly acidified with phosphoric acid until the precipitation of the enantiomerically enriched (R)-3-amino-2-benzyl-propionic acid (ee>99%) obtained by filtration

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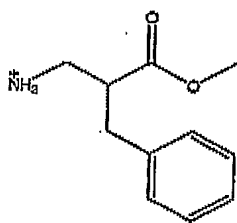
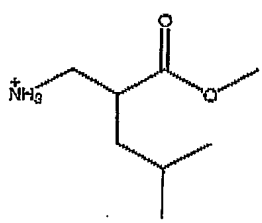
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Examples 7-14Preparation of enantiomerically enriched  $\beta^2$ -amino acid using different stereoselective hydrolytic enzymes

- 5 To a solution of substrate (200 mg) in 10 mL of sodium phosphate buffer (25 mM, pH 7.0) is added 200 mg enzyme and the resulting mixture is stirred at room temperature. The reaction is following by chiral HPLC and at approximately 50% conversion; the enantiomeric excess of the acid was measured. Corresponding E-ratios were calculated and results of the experiments are presented in table 1 below.

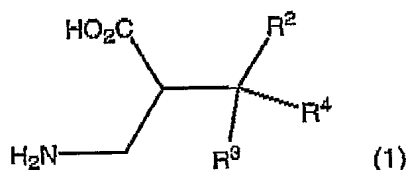
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Table 1. Preparation of enantiomerically enriched  $\beta^2$ -amino acid using different stereoselective hydrolytic enzymes

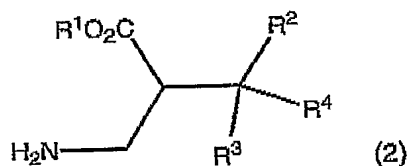
Example number	Substrate	Enzyme used	e.e. acid %	conversion %	E ratio
7		Porcine pancreatic lipase type II (L-3126, Sigma)	81	51	25
8		Alcalase 2.5L type B, PMN 4211	98	49	>300
9		<i>Bacillus licheniformis</i> protease (Pescalase, DSM-Gist)	98	49	>300
10		Alcalase 2.5L DX, PMN 4501 A-16 933131	97	49	>200
11		Alcalase 2.5L DX, PMN 4501 A-16 933131	97,2	42,7	>100
12		Alcalase 2.5L type B, PMN 4211	97,4	42,9	>100
13		<i>Bacillus licheniformis</i> protease (Pescalase, DSM-Gist)	75,3	46,2	14
14		Porcine pancreatic lipase type II (L-3126, Sigma)	99,0	49,2	>300

CLAIMS

1. Process for the preparation of an enantiomerically enriched  $\beta^2$ -amino acid of formula 1



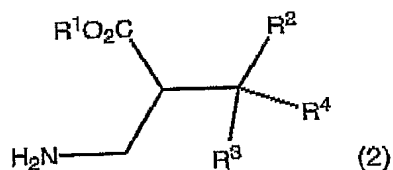
wherein  $R^2$ ,  $R^3$  and  $R^4$  each independently stand for H, an optionally substituted (hetero)aryl, an optionally substituted alkyl,  $OR^5$ ,  $CO_2R^6$ ,  $C(O)R^7$ ,  $SR^8$ ,  $NR^9R^{10}$ ,  $OC(O)R^{11}$  wherein  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^8$ ,  $R^9$ ,  $R^{10}$  and  $R^{11}$  each independently stand for H, an optionally substituted alkyl or for an optionally substituted (hetero)aryl and wherein  $R^2$  and  $R^3$ ,  $R^2$  and  $R^4$  or  $R^3$  and  $R^4$  may form a ring together with the carbon atom to which they are attached, comprising the steps of reacting a stereoselective hydrolytic enzyme with a mixture of enantiomers of a  $\beta^2$ -amino acid ester of formula 2



15

wherein  $R^1$  stands for an optionally substituted alkyl and wherein  $R^2$ ,  $R^3$  and  $R^4$  are as defined above and collecting the resulting enantiomerically enriched  $\beta^2$ -amino acid of formula 1.

2. Process for the preparation of an enantiomerically enriched  $\beta^2$ -amino acid ester of formula 2

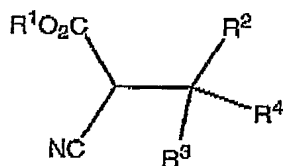


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wherein  $R^1$  stands for an optionally substituted alkyl and wherein  $R^2$ ,  $R^3$  and  $R^4$  each independently stand for H, an optionally substituted (hetero)aryl, an optionally substituted alkyl,  $OR^5$ ,  $CO_2R^6$ ,  $C(O)R^7$ ,  $SR^8$ ,  $NR^9R^{10}$ ,  $OC(O)R^{11}$  wherein  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^8$ ,  $R^9$ ,  $R^{10}$  and  $R^{11}$  each independently stand for H, an optionally substituted alkyl or for an optionally substituted (hetero)aryl and wherein  $R^2$  and  $R^3$ ,  $R^2$  and  $R^4$  or  $R^3$  and  $R^4$  may form a ring together with the carbon atom to which they are attached, comprising the steps of reacting a stereoselective hydrolytic enzyme with a mixture of enantiomers of a  $\beta^2$ -amino

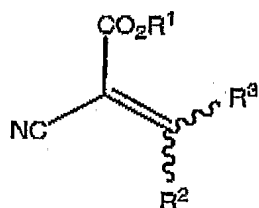
acid ester of formula 2, wherein  $R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$  are as defined above and collecting the remaining enantiomerically enriched  $\beta^2$ -amino acid ester of formula 2.

3. Process according to claim 1 or claim 2, wherein the stereoselective hydrolytic enzyme is an enzyme from the enzyme classification group EC 3.1.1, 3.4.21, 3.4.22 or 3.4.23.
4. Process according to any one of claims 1-3, wherein the stereoselective hydrolytic enzyme has an E-ratio  $> 5$ .
5. Process according to any one of claims 2-4, wherein the collected remaining enantiomerically enriched  $\beta^2$ -amino acid ester is further hydrolysed in a manner known per se.
6. Process according to any one of claims 1-5, wherein the  $\beta^2$ -amino acid ester of formula 2 is prepared by reduction of the corresponding nitrile of formula 3



(3)

7. Process according to claim 6, wherein the nitrile of formula 3, wherein  $R^1$ ,  $R^2$  and  $R^3$  are as defined above and wherein  $R^4$  stands for H is prepared by reduction of the corresponding nitrile of formula 4,



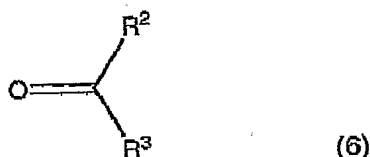
(4)

8. Process according to any one of claims 1-5, wherein the  $\beta^2$ -amino acid ester of formula 2, wherein  $R^4$  stands for H and  $R^1$ ,  $R^2$  and  $R^3$  are as defined above is prepared by reduction of the corresponding nitrile of formula 4, wherein  $R^1$ ,  $R^2$  and  $R^3$  are as defined above with a suitable reducing agent and optionally in the presence of a suitable catalyst.
9. Process according to claim 6, wherein the nitrile of formula 3, wherein  $R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$  are as defined above is prepared from the corresponding nitrile of

-16-

formula 4, wherein  $R^1$ ,  $R^2$  and  $R^3$  are as defined above by introduction of  $R^4$  via nucleophilic 1,4-addition using a suitable nucleophile.

10. Process according to any one of claims 7-9, wherein the nitrile of formula 4, wherein  $R^1$ ,  $R^2$  and  $R^3$  are as defined above is prepared by condensation of a ketone or aldehyde of formula 6

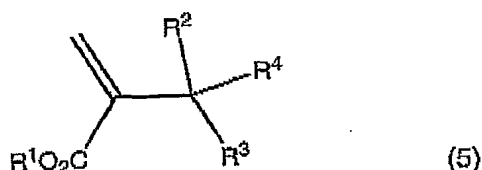


wherein  $R^2$  and  $R^3$  are as defined above and a nitrile of formula 7



wherein  $R^1$  is as defined above, in the presence of a suitable base or a dehydrating reagent.

11. Process according to any one of claims 1-5, wherein the  $\beta^2$ -amino acid ester of formula 2, wherein  $R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$  are as defined above is prepared by reacting  $NH_3$  or an  $NH_3$ -analogue with the 2-substituted acrylic acid ester of formula 5



20 wherein  $R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$  are as defined above.

12. Process according to any one of claims 1-11, wherein the enantiomerically enriched  $\beta^2$ -amino acid (ester) prepared according to a process of any one of claims 1-11 is further converted into a pharmaceutically active ingredient.

13. Process according to claim 12, wherein the pharmaceutically active ingredient is formulated into a pharmaceutical composition comprising the pharmaceutically active ingredient and an excipient.

ABSTRACT

- The invention relates to a process for the preparation of a
- 5 enantiomerically enriched  $\beta^2$ -amino acid comprising the steps of reacting a stereoselective hydrolytic enzyme with a mixture of enantiomers of a  $\beta^2$ -amino acid ester and collecting the resulting enantiomerically enriched  $\beta^2$ -amino acid. The invention also relates to a process for the preparation of an enantiomerically enriched  $\beta^2$ -amino acid ester comprising the steps of reacting a stereoselective hydrolytic
- 10 enzyme with a mixture of enantiomers of a  $\beta^2$ -amino acid ester and collecting remaining enantiomerically enriched  $\beta^2$ -amino acid ester.



